Impact of cooking on the bioactive compounds and antioxidant activity of gherkin (*Cucumis anguria* L.)

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ARTICLE INFO	ABSTRACT
	Despite the high consumption of Japanese gherkin in the Northeast of Brazil, very few studies with this vegetable are found in the literature. As it is consumed raw or after cooking, being mainly consumed cooked, there was the interest to know if cooking could influence bioactive compounds content and antioxidant activity since high temperatures are related to benefits losses in food. This research aimed to compare the content of bioactive compounds and the antioxidant activity between *in natura* and cooked Japanese gherkin. Gherkins were submitted to quantification of carotenoids, anthocyanins, vitamin C and phenolic compounds, in addition to the antioxidant activity analyses through the ferric reducing antioxidant power (FRAP) and the DPPH free radical scavenging methods. After cooking, carotenoids (from 4.90 to 4.59 μg.g^{-1}) and phenolic compounds (from 17.07 to 14.95 mg.100 g^{-1}) content decreased, but with no significant difference between *in natura* and cooked samples. As for the antioxidant activity, the *in natura* gherkin showed a higher value (3.31 μM Ferrous Sulfate.g^{-1}) than the cooked one (1.96 μM Ferrous Sulfate.g^{-1}) for FRAP method, while for DPPH method no difference between the samples was detected. Anthocyanin and vitamin C were not detected in gherkins. We concluded that, in general, cooking did not affect the bioactive compounds of gherkins and that antioxidant activity increased. **Keywords:** Gherkin, phenolic compounds, antioxidant activity, vegetable, carotenoids, vitamin C.

Introduction

Gherkin (*Cucumis anguria* L.) is indigenous to East Africa and belongs to the family Cucurbitaceae (Souza Neta et al., 2018). It can be found, in some cases, growing subspontaneously in other crops, and weighs, on average, 30 g. The traditional varieties are "Maxixe do Norte" (with thorns) and "Maxixe Japonês" (without thorns) (Brasil, 2010).

Gherkin fruits are much appreciated in Brazilian cuisine, especially in the Northeast, even though it is considered a secondary crop in that region (Oliveira et al., 2014). It is used in a dish called "maxixada"; however, it can also be consumed *in natura* in salads, as well as cooked in soups, stir-fried (Oliveira et al., 2015), and canned (pickles) (Nascimento, Nunes & Nunes, 2011).

It is best developed in the warm seasons because the cold weather and mild frosts are harmful to the vegetable, thus in warm regions, it grows throughout the whole year. It is farmed mostly in the north and northeast regions of Brazil, and only a little in the south-central region of the country but it is highlighted in other regions such as north of Minas Gerais, Rio de Janeiro, and São Paulo (Oliveira et al., 2010).

In the last few years, it has been proved that free radicals are responsible for aging, and the onset of degenerative diseases related to aging; among them are cancer, cataract, cardiovascular diseases, and others. To eliminate free radicals, and thus reduce the risk of certain diseases, the consumption of antioxidants becomes necessary. However, synthetic antioxidants have toxic effects for some extensions. Therefore, the first choice should be the absorption of natural antioxidants from food because natural antioxidants do not just play an important role in the prevention and

adjunctive treatment of diseases but also can avoid adverse reactions to human health (Li et al., 2014).

Vegetables and fruits are recommended to reduce the occurrence of such chronic diseases because of their compounds with antioxidant activity, also called bioactive compounds (Podsedek, 2007). The phenolic compounds are among the most studied bioactive compounds; they are secondary compounds of the plants, found widely in nature. Flavonoids, tannins, and/or phenolic acids are the main phenolic compounds present in human food (Angelo & Jorge, 2007).

Carotenoids, in turn, are pigment compounds very important in human food. There is evidence that they have beneficial effects on eye health, cognitive function, and cardiovascular health, may help to reduce the risk of some types of cancer, and can be a precursor for vitamin A. Carotenoids are also used as colorants in food, beverages, and pharmaceutical, varying their color from yellow to orange, but they can also be red (Eggersdorfer & Wyss, 2018).

Other important compounds are anthocyanins, which can be used as natural food colorants to replace synthetic ones, as they are not well accepted by consumers nowadays (He et al., 2015). In addition, anthocyanins are known for their biological functions, such as antioxidant and anti-carcinogen activities, and their capacity to protect the liver and enhance memory (Hwang et al., 2011). Therefore, extending the use of anthocyanin-rich extracts in the food industry is important because products with potential health benefits can be offered in addition to presenting attractive colors (He et al., 2015).

The antioxidant activity of those bioactive compounds varies according to their chemical structure and the content of those phytochemicals in the food; which can vary according to genetics, environmental conditions, ripeness degree, plant variety, among other factors (Campos et al., 2008; Melo et al., 2009).

It is known that vegetables are mostly consumed *in natura*, however, there are cases when cooking is needed and preferable; and then the bioactive compounds present in those vegetables can be modified. Those compounds can also be modified due to storage and some processing, causing loss of antioxidants or improvement of the antioxidant capacity, formation of new compounds with pro- or antioxidant activity, or even no modification at all in the concentration of the compounds (Campos et al., 2008).

Knowing that gherkin is a very consumed vegetable and of easy cultivation, it becomes important to investigate the presence of bioactive compounds and quantify them. The objective of the present study was to quantify and compare the content of bioactive compounds (phenolic compounds, carotenoids, and anthocyanins) and the antioxidant capacity of Japanese gherkin (*Cucumis anguria* L.), *in natura* and subjected to cooking because it is the most consumed form of this vegetable.

Material and Methods
Chemicals and solutions
All standards and reagents were of analytical grade. The solutions were also prepared with reagents of analytical grade.

Ascorbic Acid L (+), Diethyl Ether, Folin-Ciocalteu, Monohydrated Gallic Acid, Sodium Carbonate, Methyl Alcohol, Ethyl Alcohol, Acetone, Heptahydrated Ferrous Sulphate, and Hexahydrated Ferric Chloride were obtained from Dinâmica. TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) and DPPH (2,2-Diphenyl-1-picryl-hidrazil) were obtained from Sigma-Aldrich.

Sampling
The vegetable was supplied from a local market in the city of Limoeiro do Norte, in the state of Ceará. A sample of 1 kg was collected and taken to the Laboratory of Food Chemistry of the Federal Institute of Education, Science and Technology of the State of Ceará (IFCE), under refrigeration, in a thermal box and protected from light. Soon after that, the samples were ground, and the analyzes of phenolic compounds, carotenoids, anthocyanins, and antioxidant activity took place. All the analyzes were made in the *in natura* gherkin and the gherkin subjected to a 10-minute boiling (100°C), in triplicate.

Analysis of total carotenoids
The analysis of total carotenoids followed the methodology described by Rodriguez-Amaya (1997). Around 6 g of each sample was used. Aiming to express it in lutein, the readings of the carotenoids sample, recovered by diethyl ether, were made in a spectrophotometer UV-VIS (190-1,100 nm) (FEMTO 600 Plus), at 445 nm, the proper wavelength for the reading of carotenoids in such conditions.

Analysis of anthocyanins
The anthocyanins from the gherkin's samples were quantified according to Teixeira, Stringheta & Oliveira (2008), using the single pH method adapted from Fuleki & Francis (1968a, 1968b). The absorbance readings of the samples were made in a spectrophotometer UV-VIS (190-1,100 nm) (FEMTO 600 Plus), at 535 nm, and the results were expressed in mg.100 g^{-1}. The

extinction coefficient ($E^{1\%}_{1cm}$) used for the single pH method (pH 2.0) was 982.

Analysis of vitamin C

The vitamin C content was analyzed by spectrophotometry UV-VIS (190-1,100 nm) (FEMTO 600 Plus), through an ascorbic acid calibration curve, as described by Pearson & Cox (1976).

Preparation of the extract for the analysis of phenolic compounds and antioxidant capacity

For obtaining the extracts, 20 g of the samples was weighed in a beaker, and 40 mL of 50% methanol (v/v) was added. After homogenization, the mixture was allowed to stand for one hour at room temperature (25°C). After that, the solution was centrifuged (Eppendorf 5804) at 5000 rpm for 15 minutes, and the supernatant was filtered to a 100 mL volumetric flask. The residue was added acetone 70% (v/v) and the procedure above was repeated. The supernatant was collected and added to the same flask as the first supernatant. Then, distilled water was added to complete the volume of the flask, obtaining the extract (Larrauri, Rupérez, & Saura-Calixto, 1997).

Total phenolic compounds

Total phenolic compounds in the samples were quantified following the methodology adapted from Larrauri, Rupérez & Saura-Calixto (1997) and Obanda & Owuor (1977).

An aliquot of 1.0 mL of the extract, 1.0 mL of Folin-Ciocalteau reagent, 2.0 mL of 20% sodium carbonate solution, and 2.0 mL of distilled water was placed in test tubes. The mixture was homogenized and then allowed to rest for 30 minutes at room temperature in the dark. Afterward, the absorbance was read in a spectrophotometer (FEMTO 600 Plus) at 700 nm. The blank consisted of the same mixture prepared for the samples, replacing the extracts with water. The concentration was calculated using a standard curve of gallic acid (0 to 50 μg.mL^{-1}) and the results were expressed in mg equivalent of gallic acid.100 g^{-1}.

Total antioxidant activity by the Ferric Reducing Antioxidant Power (FRAP) method

The ferric reducing capacity was measured following the methodology described by Rufino et al. (2010). Firstly, the FRAP reagent was prepared by the mixture of 25 mL of 0,3 M acetate buffer solution (pH 3,6), 2.5 mL of 10 mM TPTZ solution, and 2,5 mL of 20 mM ferric chloride.

In test tubes, 90 μL of three different dilutions of the extract, 270 μL of distilled water, and 2.7 mL of FRAP reagent were placed. The mixture was homogenized and then allowed to rest in the dark for 30 minutes at 37°C. Afterward, the absorbance was read in a spectrophotometer (FEMTO 600 Plus) at 595 nm. The blank consisted of FRAP reagent. The antioxidant activity was calculated using a standard curve of ferrous sulfate (50 to 2000 μM) and the results were expressed in μM Ferrous Sulfate g^{-1}.

Determination of the total antioxidant activity through DPPH free radical scavenging method

This analysis was made following the methodology described by Rufino et al. (2010).

In test tubes, 0.1 mL of three different dilutions of the extract and 3.9 mL of DPPH free radical solution were placed. The mixture was homogenized, and the absorbance was read in a spectrophotometer (FEMTO 600 Plus) at 595 nm after the stabilization of the absorbance. The blank consisted of methyl ether. The antioxidant activity was calculated using a standard curve of DPPH free radical (50 to 2000 μM) and the results were expressed in EC_{50} (kg.g^{-1}), which corresponds to the amount of sample necessary to have a 50% reduction from the initial quantity of DPPH free radical.

For the bioactive compounds, the results were expressed on a dry basis, to properly evaluate the real retention of these compounds after the thermal treatment applied; and on a wet basis, to better represent the content of these compounds present in the food in the way it is consumed. For the antioxidant activity, in turn, the results were only expressed on a wet basis.

Statistical analysis

All the analyzes were made in triplicate and the results were expressed as mean and standard deviation. The Student's t-test was used to compare the two means obtained with the aid of the software Statistica 7.0 considering a statistical difference with a 5% level of significance.

Results and Discussion
Carotenoids, anthocyanins, and vitamin C

Table 1 shows the results obtained from the carotenoids, anthocyanins, and vitamin C analyzes. The contents were not statistically different (p $\geq$ 0.05) between the samples.

Table 1. Results of the analyzes of carotenoids, anthocyanins, and vitamin C for *in natura* and cooked gherkin. Same letters in the column do not differ from each other (p ≥ 0.05) by Student's t-test. [†]μg lutein.g[-1]; [‡]N.D. = Not detectable. Font: Mendes et al. (2020)

Sample	Carotenoids (μg.g^{-1})[†]		Anthocyanins[‡]	Vitamin C[‡]
	Wet basis	Dry basis		
Gherkin *in natura*	4.90 ± 0.12 [a]	97.93 ± 2.32 [a]	N.D.	N.D.
Cooked Gherkin	4.59 ± 0.22 [a]	91.73 ± 4.39 [a]	N.D.	N.D.

Regarding carotenoids, low values were obtained for both samples. Yet, the whole fruit does not have a considerable amount of this pigment, differently from what was reported by other authors referring to isolated parts of the fruit and the plant. Bernhardt & Schlich (2006) state that the composition of carotenoids varies according to their distribution in different parts of the vegetable, among other reasons. According to the research of Dzomba & Mupa (2012), the carotenoid content in leaves of wild gherkins corresponded to 47.8%, among several phytochemicals (on a dry basis), being the most abundant pigment in that study.

Compared to other vegetables, such as the pumpkin cultivar (*Cucumis maxima*, family Cucurbitaceae) studied by Kandlakunta, Rajendran & Thingnganing (2008), we can notice that gherkin has 4.6 times the concentration of carotenoids found by the quoted authors, on a dry basis.

Different from what was observed in the present research, when analyzing the influence of several thermal processes in the retention of bioactive compounds and antioxidant activity of chopped cauliflower, Ahmed & Ali (2013) found that cooking for 6 minutes in boiling water resulted in a great loss of phenolic compounds, carotenoids, vitamin C, and antioxidant activity.

According to Bernhardt & Schlich (2006), cutting the vegetable and exposing it to high temperatures can lead to cellular breakage and subsequent dissociation of some phenolic compounds previously linked to structures such as lignin and polysaccharides, for instance. Thus, if carotenoids can behave similarly, the fact that the vegetable was cooked when it was still whole might have contributed to the absence of significant difference (p ≥ 0.05) between *in natura* and cooked gherkin.

As for vitamin C and anthocyanins, according to Oliveira et al. (2016), pumpkin, which belongs to the same family as the gherkin, has, on average, 2.12 mg of vitamin C.100g[-1]. Daiutu et al. (2012), when subjecting the pumpkin to immersion cooking, found 1.06 mg of vitamin C.100g[-1], whereas Dick et al. (2016) found 2.20 mg.kg^{-1} in cucumber leaf extract (same family as the gherkin), and Dzomba & Mupa (2012) noticed that anthocyanins accounted for only 10% of the bioactive compounds found in gherkin leaves.

No studies were found reporting the presence of anthocyanins in the gherkin fruit. In the present study, anthocyanin and vitamin C contents were lower than the detectable limit using the adopted methodologies, indicating that gherkin has very low concentrations of these compounds.

Phenolic compounds and antioxidant activity

Table 2 shows the results obtained from the analyzes of phenolic compounds and antioxidant capacity by the FRAP and DPPH methods.

Table 2. Results of the analyzes of phenolic compounds and antioxidant capacity by the DPPH and FRAP methods *in natura* and cooked gherkin. Same letters in the column do not differ from each other (p ≥ 0.05) by Student's t-test. W. B.: Wet Basis / D. B: Dry Basis. [†]Expressed in Equivalent of Gallic Acid; [‡]Ferrous Sulfate; [§]Expressed as Kg sample.g[-1] DPPH. Font: Mendes et al. (2020)

ample	Phenolic Compounds (mg EAG[†].100 g^{-1})		FRAP (μM SF[‡].g[-1])	DPPH radical scavenging (EC$_{50}$)[§]
	W. B.	D.B.	W. B.	W. B.
In natura gherkin	17.07 ± 0.44 [a]	378.54 ± 9.80 [a]	1.96 ± 0.14 [b]	101.60 ± 4.49 [a]
Cooked gherkin	14.95 ± 1.47 [a]	320.79 ± 31.48 [b]	3.31 ± 0.27 [a]	169.87 ± 52.95 [a]

It can notice the presence of total phenolic compounds in gherkin, in both ways (*in natura* and cooked). Gill, Mahajan & Arora (2014), when evaluating the therapeutic potential of gherkin seeds sold in the market, obtained 41.25 mg.g^{-1} in ethanol extract produced from dry ground seeds, and this value was considered expressive by the authors.

The content of total phenolic compounds in gherkin, both *in natura* and cooked, is lower than in grape, which is known as being rich in these compounds. Regina et al. (2010), when analyzing grapes of the cultivar Chardonay, found results varying from 635 to 914 mg gallic acid.100 g^{-1} in barks and from 7419 to 10225 mg gallic acid.100 g^{-1} in the seeds from the pulp, on a wet basis.

The contents of phenolic compounds in the assayed samples, on a dry basis, show that cooking resulted in significant losses ($p < 0.05$) of these compounds, despite the very close values; however, in a wet basis (that better represents the concentrations of the compounds in the food during intake), no significant difference was detected ($p \geq 0.05$) regarding this parameter, inferring that the intake of the cooked gherkin does not mean a significative loss of phenolic compounds, compared to the *in natura* one. This result also shows that these compounds have certain stability when subjected to high temperatures.

The presence of phenolic compounds in food is essential, as several studies report its influence in preventing some diseases such as cancer, diabetes, cardiovascular diseases, and other aging-related diseases (Fusco et al., 2010). It shows the importance of analyzing the effect of food cooking on the content of phenolic compounds.

Vallejo, Tomás-Barberán & Garcia-Viguera (2003) and Zambrano-Moreno et al. (2015) agrees with the fact that polyphenols content can increase and shows certain stability (remains constant) when they are exposed to high temperatures, a quality that is reflected in the preservation of their antioxidant capacity when compared to the fresh vegetable. On the other hand, Achckar et al. (2013) reported that thermal decomposition is one of the main causes of reduction in the content of these compounds, in which a reaction of the phenols can occur with other compounds in warm environments, blocking their extraction.

Faller & Fialho (2009) concluded in their research, where several vegetables subjected to home thermal processes were analyzed, that changes in polyphenol levels can vary greatly depending on the vegetable. They observed that the retention or loss of polyphenols varied depending on the vegetable and the type of polyphenol present, with the hydrolysable polyphenols showing more stability than the soluble ones

In the same way, Nayak, Liu & Juming (2015) observed that food processing can affect positively and negatively the antioxidant activity, depending on factors such as food processing operations, additive and/or synergistic effects of the antioxidants in the food, food matrix, part of the vegetable where the antioxidants are located.

In the present work, we noticed that, regarding the ferric reducing power (FRAP) method, cooked gherkin showed an antioxidant activity significantly higher ($p < 0.05$) than *in natura* gherkin, which did not happen when the DPPH free radical scavenging method was used, where the cooked gherkin did not show any significant difference ($p \geq 0.05$).

Tiveron et al. (2013), analyzing 23 vegetables in the Brazilian market, noticed that the lowest values of antioxidant activity by the FRAP method were in celery, leek, carrot, turnip, and green beans; all of them with values near 10 µM of ferrous sulfate.g^{-1}. This value was greater than what was found *in natura* or cooked gherkin, pointing to a probable low ferric reducing power of this vegetable.

In this research, the greater iron reducing power in cooked gherkin can be attributed to both the fact that a compound with a redox potential lower than the pair Fe^{3+}/Fe^{2+} may have been released during cooking, as it is a limitation of the method according to Magalhães et al. (2008), and the fact that phenolic compounds were released (Bernhardt & Schlich, 2006), making possible a better antioxidant activity by FRAP method in cooked gherkin.

The EC_{50} results, in both samples of gherkin, point to a low antioxidant capacity through DPPH method when compared to the extracts of different parts melon (*Cucumis melo*), which is a fruit of the same genus as gherkin, in the study of Ismail et al. (2010). The authors found EC_{50} varying from 1.52 to 25.44 mg.mL^{-1}, and the values for standard antioxidant compounds such as ascorbic acid and α-tocopherol were 0.02 and 0.06 mg.mL^{-1}, respectively. We should emphasize that low EC_{50} values point to a great capacity of DPPH free radical scavenging because it corresponds to the necessary quantity of sample to reduce in half the initial concentration of DPPH radical.

Interesting results were reported in the studies of Hernández-Carranza et al. (2016), who stated that higher temperatures (60 ºC) were more efficient for the extraction of phenolic compounds, flavonoids, and antioxidant capacity (DPPH and FRAP) in byproducts of apple, orange, and banana processing; it can therefore be inferred that warming certain food helps at least the measurement of some bioactive compounds and antioxidant activity. Dewanto et al. (2002) also found higher antioxidant activity in processed tomatoes than in raw tomatoes because of the release of bound phenolic compounds in the food matrix.

Even though the difference was not significant (p ≥ 0.05) between the concentrations of phenolic compounds in the gherkin samples (wet basis), the antioxidant activity by FRAP method was significantly higher (p < 0.05) in cooked gherkin. On the other hand, the capacity for stabilization of the DPPH radical was not influenced by the cooking process (p ≥ 0.05). Thus, the consumption of cooked gherkin is recommended for better antioxidant action in the body.

According to literature, there is a positive correlation between the concentration of phenolic compounds in food and its antioxidant capacity; this means the more the content of these compounds the more the antioxidant activity (Dudonné et al., 2009; Pandey & Rizvi, 2009; Vieira et al., 2011). However, Tiveron et al. (2013) also noticed different results. The authors obtained a correlation between the content of phenolic compounds and the antioxidant activity that varied depending on the method: DPPH (R^2 = 0.34); FRAP (R^2 = 0.686).

According to Thaipong et al. (2006), the FRAP methodology is the one that presents more correlation with the vitamin C content. We can notice the same in this study, as it was not possible to detect the content of vitamin C by the methodology used, and the antioxidant activity by FRAP showed low values.

Conclusion

This study makes contributions in the areas of bioactive compounds and food processing, as the characterization of gherkin (*Cucumis anguria*) regarding the content of bioactive compounds and the antioxidant activity, and the effect of cooking in its bioactive properties is not reported in the literature.

Gherkin of the Japanese variety proved to be a minor source of phenolic compounds and presented a low concentration of carotenoids and non-detectable contents of anthocyanins and vitamin C.

The cooking process affected the phenolic compounds leading to their reduction; however, the cooked gherkin intake provides a similar content of these compounds when compared to the raw gherkin.

Antioxidant activity increased in terms of FRAP, whereas the DPPH free radical scavenging capacity remained constant after the application of thermal treatment. The intake of cooked gherkin is recommended to obtain more benefits from the antioxidant compounds in it.

Further studies about other cooking conditions (time/temperature) and other methods of thermal processing should be carried out to provide more information about the stability of the bioactive compounds and the antioxidant activity of this vegetable.

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